Tea, Coffee, and Cocoa as Ultraviolet Radiation Protectants for the Beet Armyworm Nucleopolyhedrovirus

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ABSTRACT The addition of 1% (wt:vol) aqueous extracts of cocoa (*Theobroma cacao* L.) (Malvales: Malvaceae), coffee (*Coffea arabica* L.) (Gentianales: Rubiaceae), and green and black tea (*Camellia sinensis* L.) (Ericales: Theaceae) provided excellent UV radiation protection for the beet armyworm, *Spodoptera exigua* (Hübner) (Lepidoptera: Noctuidae), nucleopolyhedrovirus under laboratory conditions. Aqueous extracts of coffee, green tea, and black tea at 0.5% provided 85–100% UV protection, whereas cocoa provided 50% UV protection. Epigallocatechin gallate (EGCG), a component of green tea, and caffeine, a component of tea and coffee, also were tested as UV protectants. Both compounds were ineffective when tested alone. When EGCG and caffeine were combined, UV protection increased in a synergistic manner, but <35% of the original virus activity was maintained. This study demonstrated that coffee was comparable to green tea and black tea as a UV protectant. Further studies should be conducted to optimize their use in biopesticide formulations.

KEY WORDS nucleopolyhedrovirus, *Spodoptera exigua*, UV radiation, cocoa, coffee

Baculoviruses are attractive biocontrol agents against lepidopterous pests because of their specificity and safety (Burges and Jones 1986). Despite the advantages of using these environmentally compatible viruses as microbial control agents, their use has been limited. One of their major limitations is their sensitivity to solar radiation, especially in the UV portion of the solar spectrum (David 1969, Dunkle and Shasha 1989, Jones et al. 1993, Shapiro et al. 2008). For the past 30+ yr, plant-derived materials have been tested as UV protectants for insect viruses (Ignoffo and Batzer 1971, Batzer and Ignoffo 1978, Behle et al. 2003, Arthurs et al. 2006, El Salamouny et al. 2009). One of the most widely used and effective UV protectants has been lignin, a natural plant polymer that is found in all vascular plants and trees. The lignin obtained from hemlock bark was developed by IMC Corporation, Libertyville, IL, and named IMC-90001 or Shade. Several patents have been issued for lignosulfates as UV protectants for biopesticides and interest in lignins for use with biocontrol agents still continues (Tamez-Guerra et al. 2000, Arthurs et al. 2006, Peng and Argyropoulos 2007).

For more than a decade, we have been investigating the use of plant-derived materials as pest control agents (Abudulai et al. 2001), virus enhancers (Shapiro et al. 2007a,b), and sunlight protectants/sun screens (Shapiro et al. 2008, El Salamouny et al. 2009). Recently, we showed that 1% water extracts of green tea provided almost complete protection for the beet armyworm nucleopolyhedrovirus (SeMNPV) after UVB radiation in laboratory tests. Under field conditions, 1 and 5% of green tea extracts were ineffective as UV protectants. At a 10% concentration, some UV protection was provided and UV protection further increased at 20 and 30% in a concentration-dependent manner (Shapiro et al. 2008). Subsequently, we demonstrated that black tea was also an excellent UV protectant for SeMNPV (El Salamouny et al. 2009). Our current study was undertaken to determine whether cocoa and coffee were as effective as green tea and black tea as UV protectants for SeMNPV. In addition, we determined whether two phenolics found in coffee, tea, and cocoa, namely, epigallocatechin gallate (ECGC) and caffeine, were as effective as the complete beverages. The goal of our studies is to obtain the most effective plant-derived material for protecting SeMNPV, and possibly other insect microbes, from degradation by sunlight.

Materials and Methods

Insects and Virus Inoculum. The insect used was a colonized strain of the beet armyworm established and maintained by USDA–ARS, Tifton, GA. Larvae were reared on the Multiple Species diet (Southland Products, Inc., Lake Village, AR) under laboratory conditions. The SeMNPV for beet armyworm, regis-

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tered as Spod-X, was obtained from Certis USA, Columbia, MD.

Extracts. Four commonly used beverages were tested as UV protectants: black tea (Lipton, Englewood Cliffs, NJ), green tea (Uncle Lee's Inc., South El Monte, CA), caffeinated coffee (Piggly Wiggly Corporation, Memphis, TN), and cocoa (Hershey, Hershey, PA). Each was obtained as a powder, and 1 g of each was blended in 99 g of distilled water and then filtered through coarse cheese cloth. Subsequent dilutions of each beverage were made to obtain 0.5 and 0.1% concentrations. The filtrates were stored at 4°C until used. In addition to the four beverages, EGCG, and caffeine obtained from Sigma-Aldrich, St. Louis, MO, as technical powders were tested alone or in combination to determine their effectiveness as UV protectants. Each powder was weighed and added to distilled water at the standard average concentration equal to that found in 1 g of green tea was determined by high-performance liquid chromatography (HPLC) as described below.

UV Radiation. The UV radiation was provided by two UVB tubes (each tube: 15 W, 382 mm; Fotodyne, Inc., New Berlin, WI), which were mounted in parallel within a Pelco UV-2 cryo chamber (Ted Pella, Inc., Redding, CA) 203.2 mm above the test dishes. (Please refer to Shapiro and Domek 2002 for radiation emission profiles.) The UVB radiation treatments exposure time was for 15, 30, or 300 min.

Exposure of SeMNPV to UVB Radiation. Preliminary bioassays were conducted to determine which virus concentration caused 90–95% beet armyworm mortality before UV radiation. As a result of these assays, SeMNPV was diluted in distilled water (standard) or a test solution to obtain a final concentration of 5×10^5 viral occlusion bodies (OBs) per ml. Four ml of virus suspension was pipetted into a 60- by 15-mm glass dish (Thermo Fisher Scientific, Waltham, MA) and was exposed to UVB for 15 (Table 1), 30, or 300 min (Table 2). After radiation, the volume was determined and distilled water was added to each dish to replace water lost by evaporation. Lids were then placed on all dishes, and dishes were stored at 4°C until used. When dishes were removed from the refrigerator, 0.1 ml of virus suspension (e.g., SeMNPV/water and SeMNPV/test material) (=37.4 OB/mm^2 of diet surface area) was applied to the diet surface each 30-ml cup (WL Enterprises Inc., Newark, NJ). In addition, nonirradiated SeMNPV in water also was introduced into cups as a positive control.

Five-day-old second instars were placed individually in each cup and were reared at 27°C under laboratory conditions. Tests were repeated five times with 10 larvae per treatment, 10 untreated larvae, and 10 test treatment controls per replicate. Black armyworm mortality was assessed initially at day 5 and every 2–4 d thereafter until day 14, when the test was terminated. The percentage of original activity remaining (% OAR) was used as the basis of UV protection and was based upon virus-caused mortality before and after radiation (Ignoffo and Batzer 1971, Ignoffo et al. 1977).

Table 1. Mortality of beet armyworm fed SeMNPV that was exposed to UV for 15 min with and without beverages

Treatment[a]	UV exposure[b] (min)	Mortality[c] ± SE (%)	OAR[d] ± SE (%)
NPV only	0	96.0 ± 2.45a	100.0 ± 2.55a
NPV only	15	6.0 ± 2.45c	6.3 ± 2.55d
Green tea, 1.0%	15	94.0 ± 2.45a	97.9 ± 2.55a
Green tea, 0.1%	15	20.0 ± 5.48bc	20.8 ± 5.71bc
Black tea, 1.0%	15	96.0 ± 2.45a	100.0 ± 2.55a
Black tea, 0.1%	15	16.0 ± 5.10bc	16.7 ± 5.31cd
Cocoa, 1.0%	15	96.0 ± 4.00a	100.0 ± 4.17a
Cocoa, 0.1%	15	30.0 ± 3.16b	31.3 ± 3.29b
Coffee, 1.0%	15	94.0 ± 4.00a	97.9 ± 4.17a
Coffee, 0.1%	15	30.0 ± 7.07b	31.3 ± 7.37b

[a] NPV used at final concentration of 37.4 OBs/mm^2 of diet surface. Five replicates; 10 larvae per treatment per replicate; 10 untreated control larvae per replicate; 10 beverage-treated larvae per replicate.

[b] Virus was exposed to UVB/UVB irradiation (15 min) in deionized water (standard) or in beverage (1.0%, 0.1%).

[c] Means within columns with the same letter are not significantly different ($P > 0.05$) by LSD.

[d] OAR = original activity remaining; mortality on a given treatment ÷ mean mortality on unexposed virus × 100.

UV Absorbance. UV absorbances of green tea, black tea, cocoa, and coffee were determined according to the method used previously by S.E. et al. (unpublished data). Absorbances of 1% beverage solutions were analyzed using a spectrophotometer (SpectraMax Plus384, Molecular Devices, Sunnyvale, CA) in the range of 190–600 nm.

HPLC. The concentrations of EGCG and caffeine in each beverage UVB radiation were quantified based on pure standards (Sigma-Aldrich) by using a reversed phase HPLC system (Shimadzu Corporation, Kyoto, Japan) equipped with a SCL-10A system controller, SIL-10AD auto injector, DGU-14A degasser, LC-10AT pump, and SPD-M10AT UV/VIS photodiode array detector set at 280 nm.

Table 2. Mortality of BAW fed SeMNPV that was exposed to UV for 300 min with and without beverages

Treatment[a]	UV exposure[b] (min)	Mortality[c] ± SE (%)	OAR[d] ± SE (%)
NPV only	0	94.0 ± 2.54a	100.0 ± 2.61ab
NPV only	300	0.0 ± 0.00d	0.0 ± 0.00e
Green tea 1.0%	300	88.0 ± 4.90ab	93.6 ± 5.21abc
Green tea 0.5%	300	88.0 ± 5.83ab	93.6 ± 6.20abc
Black tea 1.0%	300	94.0 ± 4.00a	100.0 ± 4.26ab
Black tea 0.5%	300	80.0 ± 3.16b	85.1 ± 3.36c
Coffee 1.0%	300	90.0 ± 5.48ab	95.7 ± 5.83abc
Coffee 0.5%	300	96.0 ± 2.45a	102.1 ± 2.61a
Cocoa 1.0%	300	84.0 ± 5.10ab	89.4 ± 5.42bc
Cocoa 0.5%	300	46.0 ± 2.45c	48.9 ± 2.61d

[a] NPV was used at final concn of 37.4 OBs/mm^2 of diet surface. Five replicates; 10 larvae per treatment per replicate; 10 untreated control larvae per replicate; 10 beverage-treated larvae per replicate.

[b] Virus was exposed to UVB/UVB irradiation in deionized water (standard) or in beverage filtrate (1.0 and 0.5%).

[c] Means within columns with the same letter are not significantly different ($P > 0.05$) by LSD.

[d] OAR, original activity remaining; mortality on a given treatment ÷ mean mortality on unexposed virus × 100. Means within columns with the same letter are not significantly different ($P > 0.05$) by LSD.

Each beverage solution was mixed well, and three replicates of each solution were filtered through 0.45-μm PTFE filters (Nalgene, Rochester, NY), and 20 μl of each replicate sample was injected to the HPLC system. A Nova-Pak C18 analytical column (Waters, Milford, MA) with the respective guard column was used to separate EGCG and caffeine at room temperature, with a flow rate of 1 ml/min. The eluents used were 2% acetic acid in water for mobile phase A, and 100% acetonitrile for mobile phase B with a total running time of 65 min per sample. The caffeine and EGCG were separated using a linear gradient of mobile phase B initially 100%; 0–50 min 31% B; 50–65 min 100% B to equilibrate the system for the next injection. All the solvents used were HPLC grade, filtered, and degassed before use. Calibration curves of the standards and their concentrations in each beverage sample based on the standards were obtained using Shimadzu 7.2 software (Shimadzu Corporation). The concentration of EGCG or caffeine in each beverage was calculated and expressed as a percentage of dry weight.

Statistical Methods. In all tests of SeMNPV exposed to UV with or without protectants, mortality was calculated as the number of dead larvae in a given replicate of a given treatment divided by 10. OAR was calculated as mortality on a given treatment divided by the mean mortality on the treatment of SeMNPV only, without UV exposure, in each respective experiment. Mortality data were normalized by arcsine $\sqrt{\%}$ transformation. OAR was not so transformed because it is possible for some observations to be >100%. Combinations of beverage and concentration or exposure times, including SeMNPV only, were treated as treatments. These data were then analyzed by analysis of variance (ANOVA), and means were separated by the least significant difference (LSD) test (SAS Institute 2008). In addition, for the test of EGCG and caffeine, those treatments of SeMNPV with a protectant were analyzed factorially with protectant and concentration as main effects. Data on the EGCG and caffeine content of beverages were analyzed by ANOVA with means separation by LSD.

Results

UVB Radiation: Beverages, 15 min. Treatment significantly affected mortality ($F_{9,\ 40} = 40.38$; $P < 0.0001$) and OAR ($F_{9,\ 40} = 93.05$; $P < 0.0001$). All beverages at 1% provided significantly better protection than did beverages at 0.1% (Table 1).

UVB Radiation: Beverages, 300 min. Treatment again significantly affected mortality ($F_{9,\ 40} = 28.13$; $P < 0.0001$) and OAR ($F_{9,\ 40} = 58.28$; $P < 0.0001$). In terms of both mortality and OAR, no beverage treatment except black tea and cocoa at 0.5% differed significantly from SeMNPV only without UV exposure (Table 2).

Concentration of EGCG and Caffeine in Beverages. Beverages differed significantly in concentrations of both EGCG ($F_{3,\ 8} = 7657.52$; $P < 0.0001$) and caffeine ($F_{3,\ 8} = 543.15$; $P < 0.0001$). All differences in EGCG among beverages were significant. Coffee and green tea did not differ in caffeine content but were both lower than black tea and higher than cocoa for this variable (Table 3).

Table 3. Average concentration (milligrams per gram of dry weight) of EGCG and caffeine in aqueous extracts of green tea, black tea, cocoa, and coffee determined using HPLC

	Green tea	Black tea	Cocoa	Coffee
ECGC[a]	35.27 ± 0.27[b]a	3.43 ± 0.10b	0.27 ± 0.04d	1.13 ± 0.08c
Caffeine[a]	23.38 ± 0.27b	30.27 ± 0.50a	1.58 ± 0.05c	23.90 ± 0.91b
Total	58.66	33.72	1.85	25.04

[a] Means within rows with the same letter are not significantly different ($P > 0.05$) by LSD.
[b] Average ± SE values were from three replicates per beverage.

UVB Radiation: EGCG and Caffeine, 30 and 300 min. Treatment significantly affected both mortality ($F_{8,\ 36} = 99.36$; $P < 0.0001$) and OAR ($F_{8,\ 36} = 192.52$; $P < 0.0001$). After 30-min exposure, the combination of EGCG and caffeine protected the virus better than did either component alone, but the combination did not provide complete protection (Table 4). Factorial analysis showed a significant interaction between EGCG and caffeine at a 30-min exposure for mortality ($F_{1,\ 16} = 19.85$; $P < 0.0004$) and OAR ($F_{1,\ 16} = 24.38$; $P < 0.0001$). No mortality occurred on any treatment exposed for 300 min in this experiment, so no further statistics are reported for these data.

UV Absorbance Spectra. In general, UV absorbance profiles of green tea, black tea, cocoa, and coffee were very similar, i.e., absorbance of UVB was high but declined significantly in the UVA portion of the UV spectrum (Fig. 1). However, cocoa was a good absorber of both UVB and UVA as shown in Fig. 1.

Table 4. Mortality of beet armyworm fed SeMNPV that was exposed to UV with or without EGCG and/or caffeine as UV protectants

Treatment[a]	UV exposure[b] (min)	Mortality ± SE (%)	OAR[c] ± SE (%)
NPV only	0	96.0 ± 2.45a	100.0 ± 2.55a
NPV only	30	6.0 ± 2.45c	6.3 ± 2.55cd
NPV only	300	0.0 ± 0.00d	0.0 ± 0.0d
EGCG	30	10.0 ± 3.16c	10.4 ± 3.29c
EGCG	300	0.0 ± 0.00d	0.0 ± 0.0d
Caffeine	30	0.0 ± 0.00d	0.0 ± 0.0d
Caffeine	300	0.0 ± 0.00d	0.0 ± 0.0d
ECGC + caffeine	30	36.0 ± 5.10b	37.5 ± 5.31b
ECGC + caffeine	300	0.0 ± 0.0d	0.0 ± 0.0d

[a] NPV used at final concentration of 37.4 OBs/mm^2 of diet surface. Five replicates; 10 larvae per treatment per replicate; 10 untreated control larvae per replicate; 10 ECGC- and 10 caffeine-treated larvae per replicate. EGCG and caffeine were used at the levels found in 1 g of green tea (e.g., EGCG = 35.5 ± 0.92 mg/g dry weight ± SE, caffeine = 23.39 ± 0.22 mg/g dry weight ± SE; 3 replicates; HPLC determinations).
[b] Virus was exposed to UVB/UVB irradiation (30 and 300 min) in deionized water (standard) or in test material.
[c] Means within columns with the same letter are not significantly different ($P > 0.05$) by LSD.

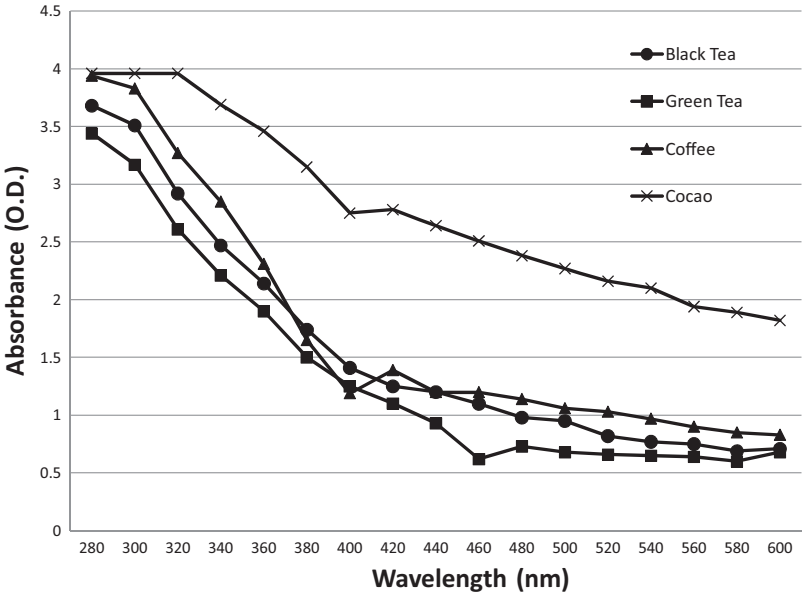

Fig. 1. UV absorbance spectra of green tea, black tea, cocoa and coffee.

Discussion

Plants have been shown to act as repellents or insecticides (George et al. 2000, Scott et al.2005, Karunamoorthi et al. 2008), to influence host susceptibility to insect viruses (Keating et al. 1988, Farrar and Ridgway 2000, Hoover et al. 2000, Ali et al. 2002) and to act as sunlight protectants (Ignoffo and Batzer 1971, Ignoffo et al. 1977, Hobbs et al. 1999). In our previous studies, six of 35 spices (Shapiro et al. 2007a) and eight of 29 medicinal herbs (Shapiro et al. 2007b) acted as virus enhancers for the gypsy moth, *Lymantria dispar* (L.), nucleopolyhedrovirus. Recently, we tested 64 medicinal herb and spice extracts and found that 27 provided almost complete protection for SeMNPV after UVA radiation. These 27 extracts were then tested under more stringent conditions (i.e., UVB for 30 and 300 min). Results of this study showed that the extracts of kudzu, peppermint, and skullcap provided almost complete UVB protection even after 300 min of UV exposure (Shapiro et al. 2008). Subsequently, we showed that black tea was an excellent UV protectant for SeMNPV under laboratory conditions (El Salamouny et al. 2009). These results were not surprising, because it had been demonstrated that green tea and black tea inhibited UVB-induced oxidative damage (Wei et al. 1999, Huang et al. 2007) and carcinogenesis (Wang et al. 1994, Lu et al. 2001, Mantena et al. 2005) in mammalian systems.

In the current study, we expanded our investigation to include two other widely used beverages with high concentrations of UV-absorbing phenolics (cocoa and coffee) (Scalbert and Williamson 2000, Alemanno et al. 2003, Koshiro et al. 2007) and antioxidants (Radtke et al. 1998, Lee et al. 2003, Pellegrini et al. 2003). All four beverages at 1.0% concentration provided excellent UV protection (>95% OAR) during a 15-min UV exposure. At a lower concentration (i.e., 0.1%), however, they provided little UV protection (<35% OAR) (Table 1). To determine which beverages would be effective as UV protectants under more severe conditions, we increased the UV exposure time from 15 to 300 min (Table 2). Even under these conditions, 1% green tea, black tea, and coffee still provided excellent UV protection (i.e., ≈90 OAR to >99.9% OAR). At the 0.5% concentration, coffee provided excellent UV protection (i.e., ≈ 95% OAR), green tea and black tea provided good UV protection (i.e., ≈80% OAR), whereas cocoa provided only fair UV protection (i.e., ≈50% OAR) (Table 2). These results were very encouraging but failed to identify which of the three beverages (coffee, green tea, and black tea) was the most efficacious UV protectant. To determine which is the most efficacious beverage we 1) tested lower concentrations under laboratory conditions and 2) conducted field tests with SeMNPV on collards, *Brassica oleracea* L. (Brassicales: Brassicaceae), to determine the efficacy of this system under natural conditions (Shapiro et al. 2008).

As stated, all four beverages are rich in UVB-absorbing phenolics (Caffin et al. 2004, Seeram et al. 2006) and are excellent sources of antioxidants (Sanbogi et al. 1998, Karori et al. 2007). For example, Caffin et al. (2004) identified 24 constituents from green tea by using HPLC. The major constituents found were EGCG (8.9% of dry weight) and caffeine (2.78% of dry weight), and they accounted for ≈12% of the total dry weight. Other compounds such as theogallin, gallic acid, theobromine, chlorogenic acid, and quercetin were also present, and 23 of the 24 chemicals had UVB absorbance peak maxima and two (e.g., kaempferol 3-rhamnosylglucoside and kaempferol glycoside) had UVA absorbance peak maxima (Caffin et al. 2004). In

black tea, the main phenolic compounds identified were EGCG, four theaflavins, as well as epicatechin gallate, theogallin, quercetin-3-rutinoside, and 4-caffeoyl quinic acid, whereas thearubingins represented an estimated 75–82% of the total phenolics (Rechner et al. 2002). In addition, caffeine, chlorogenic acid, and gallic acid also were found in black tea (Ozawa 1982, Turkmen and Sedat Velioglu 2007). In cocoa, phenolics constitute 12–18% (dry weight) in beans (Kim and Keeney 1984) and caffeine, (−)-epicatechin, (+)-catechin, quercetin, and theophylline also are found in cocoa beans (Shively and Tarka 1984, Sanbogi et al. 1998, Thomas et al. 2004). In coffee, the principal phenolics include chlorogenic acid and breakdown products such as melanoidins, caffeic acid, caffeine, and epicatechin (Radtke et al. 1998, Ramirez-Coronel et al. 2004, Bekedam et al. 2008, George et al. 2008). Because many of these chemicals are good UV absorbers and antioxidants (Pellegrini et al. 2003, Caffin et al. 2004), it was not surprising that the four beverages provided good to excellent UV protection for SeMNPV.

Previously, we demonstrated that caffeinated green tea provided greater UV protection than decaffeinated green tea but did not determine "whether maximal UV protection was due to a single compound or to a combination of chemicals in aqueous extracts of green tea" (Shapiro et al. 2008). Thus, would a single chemical or even a combination of chemicals have similar activities of a whole green tea extract that contains many different compounds acting in a additive or synergistic manner? The two compounds, EGCG and caffeine, were chosen because 1) they are the most abundant chemicals found in green tea (Song et al. 2003, Caffin et al. 2004); 2) they both have been shown to inhibit the detrimental effects of UVB radiation in mammals (Lu et al. 2000, 2007; Katiyar 2003; Conney et al. 2008; Varma et al. 2008); 3) they have antioxidant activities (Devasagayam et al. 1996, Katiyar et al. 2001, Choi et al. 2005, Karori et al. 2007); 4) their absorbance maxima is in the UVB portion of the spectrum (Mittai et al. 2003, Caffin et al. 2004, Yamauchi et al. 2008); 5) they have been found to inhibit UVB-induced damage (Lu et al. 2000, Katiyar 2003, Varma et al. 2008); and 6) they occur in green tea, black tea, cocoa, and coffee (Table 3).

In the current study, we determined the presence and amount of EGCG and caffeine in 1 g of green tea, black tea, cocoa, and coffee by using the HPLC (Caffin et al. 2004) (Table 3) and then compared the effectiveness of these compounds, alone and in combination, as UV protectants for SeMNPV at the concentration found in 1 g of dry green tea (Table 4). Although green tea contained ≈60 mg/gm dry weight of EGCG and caffeine, and cocoa contained only ≈2 mg/gm dry weight of EGCG and caffeine (Table 3), no differences occurred in their effectiveness as UV protectants (at 1%) (Tables 1 and 2), indicating that other compounds also may act as UV protectants. Moreover, when EGCG and caffeine were used alone, they were ineffective as UV protectants after UV exposures of 30 min (e.g., 0–10% OAR). After 300-min exposure, they provided no UV protection for SeMNPV. When the two compounds were combined, they seemed to act synergistically (30-min UV, ≈36% OAR) but offered no protection at a 300-min UV exposure (Table 4). If we compare these results with those obtained for green tea (30- and 300-min UV) (Table 2) and for EGCG, caffeine, and EGCG + caffeine (30- and 300-min UV) (Table 4), it is obvious that EGCG and caffeine alone or in combination were not totally responsible for the UV protection provided by an aqueous extract of green tea (≈94% % OAR) (Table 2). Thus, green tea, which contains many compounds with complex interrelations and activities, was much more effective than one or two compounds by themselves or in combination. The significant statistical interactions between EGCG and caffeine are consistent with synergism.

Because we have demonstrated that diverse plant extracts have potential as UV protectants (Shapiro et al. 2008, El Salamouny et al. 2009), we are continuing our studies in this promising area to obtain the most effective UV protectants as our goal is to obtain the most effective UV protectants as "adjuvants for insect pathogenic viruses in pest management of agriculturally important insects" (Shapiro et al. 2007a).

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References Cited

Abudulai, M., B. M. Shepard, and A. Salifu. 2001. Field evaluation on a neem (*Azadirachta indica* A. Juss)-based formulation Neemix® against *Nezara viridula* (L.) (Hemiptera: Pentatomidae) in cowpea. Int. J. Pest Manage. 49: 109–113.

Alemanno, L., T. Ramos, A. Gargadenec, C. Andary, and N. Ferriere. 2003. Localization and identification of phenolic compounds in *Theobroma cacao* L. somatic embryogenesis. Ann. Bot. (Lond.) 92: 613–623.

Ali, M. L., S. Y. Young, G. W. Felton, and R. W. McNew. 2002. Influence of the host plant on occluded virus production and lethal infectivity of a baculovirus. J. Invertebr. Pathol. 81: 158–165.

Arthurs, S. P., L. A. Lacey, and R. W. Behle. 2006. Evaluation of spray-dried lignin-based formulations and adjuvants as solar protectants for the granulovirus of the codling moth, *Cydia pomonella* (L.). J. Invertebr. Pathol. 93: 88–95.

Behle, R. W., P. Tamez-Guerra, and M. R. McGuire. 2003. Field activity and storage stability of *Anagrapha falcifera* (AfMNPV) in spray-dried lignin-based formulations. J. Econ. Entomol. 96: 1066–1075.

Bekedam, E. K., M. J. Loots, H. A. Schols, M. A. Van Boekel, and G. Smit. 2008. Roasting effects on formation mechanisms of coffee brew melanoidins. J. Agric. Food. Chem. 56: 7138–7145.

Burges, D. H., and K. Jones. 1986. Formulations of bacteria, viruses, and protozoa to control insects, pp. 33–129. *In* H. D. Burges [ed.], Formulation of microbial pesticides. Kluwer Academic Publishers, Dordrecht, The Netherlands.

Caffin, N., B. D'Arcy, L. Yao, and G. Rintoul. 2004. Developing an index of quality for Australian tea. RIRDC Publication 04/033. Rural Industries Research and Development Corp., Canberra, Australia.

Choi, Y. J., Y. J. Jeong, Y. J. Lee, H. M. Kwan, and Y. H. Kang. 2005. Epigallo-catechin gallate and quercetin enhance survival signaling in the response to oxidant-induced human endothelial apoptosis. J. Nutr. 135: 707–713.

Conney, A. H., S. Zhou, M. J. Lee, J. G. Xie, C. S. Yang, Y. R. Lou, and Y. Lu. 2008. Stimulatory effect of oral administration of tea, coffee or caffeine on UVB-induced apoptosis in the epidermis of SKH-1 mice. Toxicol. Appl. Pharmacol. 224: 209–213.

David, W.A.L. 1969. The effect of ultraviolet radiation of known wavelengths on a granulosis virus of *Pieris brassicae.* J. Invertebr. Pathol. 14: 336–342.

Devasagayam, T. P., J. P. Kamat, H. Mohan, and P. C. Kesavan. 1996. Caffeine as an antioxidant: inhibition of lipid peroxidation induced by reactive oxygen species. Biochim. Biophys. Acta 1282: 63–70.

Dunkle, R. L., and B. S. Shasha. 1989. Response of starch-encapsulated *Bacillus thuringiensis* containing ultraviolet screens to sunlight. Environ. Entomol. 18: 1035–1041.

El Salamouny, S., M. Shapiro, K. S. Ling, and B. M. Shepard. 2009. Black tea and lignin as ultraviolet protectants for the beet armyworm nucleopolyhedrovirus. J. Entomol. Sci. 44: 1–9.

Farrar, R. R., Jr., and R. L. Ridgway. 2000. Host plant effects on the activity of selected nuclear polyhedrosis viruses against the corn earworm and beet armyworm (Lepidoptera: Noctuidae). Environ. Entomol. 29: 108–115.

George, J., H. P. Bais, and G. A. Ravishankar. 2000. Biotechnological production of plant-based insecticides. Crit. Rev. Biotechnol. 20: 49–77.

George, S. E., K. Ramaiakshmi, and L. J. Mohan Rao. 2008. A perception on health benefits of coffee. Crit. Rev. Food Sci. Nutr. 48: 464–486.

Hoover, K., J. O. Washburn, and L. E. Volkman. 2000. Midgut-based resistance of *Heliothis virescens* baculovirus infection mediated by phytochemicals in cotton. J. Insect Physiol. 46: 999–1007.

Huang, C. C., W. B. Wu, J. Y. Fang, H. S. Chiang, S. K. Chen, B. H. Chen, Y. T. Chen, and C. F. Hung. 2007. (−)-Epicatechin-3-gallate, a green tea polyphenol is a potent agent against UVB-induced damage in HaCaT keratinocytes. Molecules 12: 1845–1858.

Ignoffo, C. M., and O. F. Batzer. 1971. Microencapsulation and ultraviolet protectants to increase sunlight stability of an insect virus. J. Econ. Entomol. 64: 850–853.

Ignoffo, C. M., D. L. Hostetter, P. P. Sikorowski, G. Sutter, and W. M. Brooks. 1977. Inactivation of representative species of entomopathogenic viruses, a bacterium, fungus, and protozoan by an ultraviolet light source. Environ. Entomol. 6: 411–415.

Jones, K. A., G. Moawad, D. J. McKinley, and D. Grywacz. 1993. The effect of natural sunlight on *Spodoptera littoralis* nuclear polyhedrosis virus. Biocontrol Sci. Technol. 3: 189–197.

Karori, S. M., F. N. Wachira, J. K. Wanyoko, and R. M. Ngure. 2007. Antioxidant capacity of different types of tea products. Afr. J. Biotechnol. 6: 2287–2296.

Karunamoorthi, K., A. Mulciam, and F. Wassic. 2008. Laboratory evaluation of traditional insect/mosquito repellent plants against *Anopheles arabiensis,* the predominant malaria vector in Ethiopia. Parasitol. Res. 103: 529–534.

Katiyar, S. K. 2003. Skin photoprotection by green tea: antioxidant and immunomodulatory effects. Curr. Drug Targets Immune Endocr. Metab. Disord. 3: 234–242.

Katiyar, S. K., F. Afaq, A. Perez, and H. Mukhtar. 2001. Green tea polyphenol (−)-epigallocatechin-3-gallate treatment of human skin inhibits ultraviolet radiation-induced oxidative stress. Carcinogenesis 22: 287–294.

Keating, S. T., W. G. Yendol, and J. C. Schultz. 1988. Relationship between susceptibility of gypsy moth larvae (Lepidoptera: Lymantriidae) to a baculovirus and host plant constituents. Environ. Entomol. 17: 952–958.

Kim, H., and P. G. Keeney. 1984. (−) Epicatechin content in fermented and unfermented cocoa beans. J. Agric. Food Chem. 47: 3693–3701.

Koshiro, Y., M. C. Jackson, R. Katahira, M. L. Wang, C. Nagai, and H. Ashihara. 2007. Biosynthesis of chlorogenic acids in growing and ripening fruits of *Coffea arabica* and *Coffea canephora* plants. Z. Naturforsch. 62: 731–742.

Lee, K. W., Y. J. Kim, H. J. Lee, and C. Y. Lee. 2003. Cocoa has more phenolic phytochemicals and a higher antioxidant capacity than teas and red wine. J. Agric. Food Chem. 51: 7292–7295.

Lu, Y. P., Y. R. Lou, X. H. Li, J. G. Xie, D. Brash, M. T. Huang, and A. H. Conney. 2000. Stimulatory effect of oral administration of green tea or caffeine on ultraviolet light-induced increases in epidermal wild-type p53, p21 (WAF1/CIPI), and apoptotic sunburn cells in SKH-1 mice. Cancer Res. 60: 4785–4791.

Lu, Y. P., Y. R. Lou, Y. Lin, W. J. Shih, M. T. Huang, C. S. Chang, and A. H. Conney. 2001. Inhibitory effects of orally administered green tea, black tea, and caffeine on skin carcinogenesis in mice previously treated with ultraviolet b light (high-risk mice): relationship to decreased tissue fat. Cancer Res. 61: 5002–5009.

Mantena, S. K., S. M. Meeran, C. A. Elmets, and S. K. Katiyar. 2005. Orally administered green tea polyphenols prevent ultraviolet radiation-induced skin cancer in mice through activation of cytotoxic T cells and inhibition of angiogenesis in tumors. J. Nutr. 135: 2871–2877.

Mittai, A., C. Piyathilake, Y. Hara, and S. K. Katiyar. 2003. Exceptionally high protection of photocarcinogenesis by topical application of (−)-epigallocatechin-3-gallate in hydrophilic cream in SKH-1 hairless mouse model: relationship to inhibition of UVB-induced global DNA hypomethylation. Neoplasia 5: 555–565.

Ozawa, T. 1982. Separation of the components in black tea infusion by chromatography on toyopearl. Agric. Biol. Chem. 46: 1079–1081.

Pellegrini, N., M. Serafini, B. Colombi, D. Del Rio, S. Salvatore, M. Bianchi, and F. Brighenti. 2003. Total antioxidant capacity of plant foods, beverages and oils consumed in Italy assessed by three different in vitro assays. J. Nutr. 133: 2812–2819.

Peng, P. P., and D. S. Argyropoulos. 2007. On the interaction of UV screens with the lignocellulosic matrix. Photochem. Photobiol. 71: 149–156.

Radtke, J., J. Linseisen, and G. Wolfram. 1998. Phenolic acid intake of adults in a Bavarian subgroup of the national food consumption survey. Z. Ernahrungswiss. 37: 190–197.

Ramirez-Coronel, M. A., M. Marnet, V. S. Kulli, S. Roussos, S. Guyot, and C. Augur. 2004. Characterization and estimation of proenthocyanidins and other phenolics in coffee pulp (*Coffea arabica*) by thiolysis-high performance liquid chromatography. J. Agric. Food Chem. 52: 1344–1349.

Rechner, A. R., E. Wagner, L. van Buren, F. van de Put, S. Wiseman, and C. A. Rice-Evans. 2002. Black tea represents a major source of dietary phenolics among regular tea drinkers. Free Radical Res. 36: 1127–1135.

Sanbogi, C., N. Osakabe, M. Natsume, T. Takizawa, S. Gomi, and T. Osawa. 1998. Antioxidative polyphenols isolated from *Theobroma cocoa.* J. Agric. Food. Chem. 46: 454–457.

SAS Institute. 2008. SAS 9.2. SAS Institute, Cary, NC.

Scalbert, A., and G. Williamson. 2000. Dietary intake and bioavailability of polyphenols. J. Nutr. 130: 2073S–2085S.

Scott, L. M., L. Gagnon, B. J. Sesage, B. J. Philogene, and J. T. Arnason. 2005. Efficacy of botanical insecticides from *Piper* species (Piperaceae) extracts for control of the European cockchafer (Coleoptera: Scarabaeidae). J. Econ. Entomol. 98: 845–855.

Seeram, N. P., S. M. Henning, Y. Niu, R. Lee, H. S. Scheuller, and D. Heber. 2006. Catechin and caffeine content of green tea dietary supplements and correlation with antioxidant activity. J. Agric. Food Chem. 54: 1599–1603.

Shapiro, M., and J. Domek. 2002. Relative effects of ultraviolet and visible light on the activities of the corn earworm and beet armyworm (Lepidoptera: Noctuidae) nucleopolyhedroviruses. J. Econ. Entomol. 95: 261–268.

Shapiro, M., S. El Salamouny, and B. M. Shepard. 2008. Green tea extracts as ultraviolet protectants for the beet armyworm, *Spodoptera exigua,* nuclepolyhedrovirus. Biocontrol Sci. Technol. 18: 605–617.

Shapiro, M., B. M. Shepard, and R. Lopez. 2007a. Effect of spices upon the activity of the gypsy moth (Lepidoptera: Lymantriidae) nucleopolyhedrovirus. J. Entomol. Sci. 42: 84–91.

Shapiro, M., B. M. Shepard, and R. Lopez. 2007b. Effects of medicinal herbs on the biological activity of the gypsy moth nucleopolyhedrovirus. J. Entomol. Sci. 42: 426–429.

Shively, C. A., and S. M. Tarka, Jr. 1984. Methylxanthine composition and consumption patterns of cocoa and chocolate products. Prog. Clin. Biol. Res. 158: 149–178.

Song, G., J. Lin, Q. Feng, and C. W. Huie. 2003. Extraction of catechins and caffeine from different tea leaves and comparison with micellar electrokinetic chromatography. Chin. Sci. Bull. 48: 2438–2443.

Tamez-Guerra, P., M. R. McGuire, R. W. Behle, J. J. Hamm, H. R. Sumner, and B. S. Shasha. 2000. Sunlight persistence and rainfastness of spray-dried formulations of baculovirus isolated from *Anagrapha falcifera* (Lepidoptera: Noctuidae). J. Econ. Entomol. 93: 210–218.

Thomas, J. B., J. H. Yen, M. M. Schantz, B. J. Porter, and K. E. Sharpless. 2004. Determination of caffeine, theobromine, and theophylline in standard reference material 2384, baking chocolate, using reversed-phase liquid chromatography. J. Agric. Food Chem. 52: 3259–3263.

Turkmen, N., and Y. Sedat Velioglu. 2007. Determination of alkaloids and phenolic compounds in black tea processed by two different methods in different plucking seasons. J. Sci. Food Agric. 87: 1408–1416.

Varma, S. D., K. R. Hegde, and S. Kovtun. 2008. UV-B-induced damage to the lens in vitro: prevention by caffeine. J. Ocul. Pharmacol. Ther. 24: 439–444.

Wang, Z. Y., M. T. Huang, Y. R. Lou, J. G. Xie, K. R. Reuhl, H. L. Newmark, C. T. Ho, C. S. Yang, and A. H. Conney. 1994. Inhibitory effects of black tea, green tea, decaffeinated black tea, and decaffeinated green tea on ultraviolet B light-induced skin carcinogenesis in 7,12-dimethylbenz[a]anthracene-initiated SKH-1 mice. Cancer Res. 54: 3428–3435.

Wei, H., X. Zhang, J. F. Zhao, Z. Y. Wang, D. Bickers, and M. Lebwohl. 1999. Scavenging of hydrogen peroxide and inhibition of ultraviolet light-induced oxidative DNA damage by aqueous extracts from green and black tea-superoxide-dismutase (SOD)-like activity measured by *Cypridina* luciferin analogue chemiluminescence. Free Radical Biol. Med. 26: 1427–1435.

Yamauchi, Y., A. Nakamura, I. Kohno, M. Kitai, K. Hatanaka, and T. Tanimoto. 2008. Simple and rapid UV spectrophotometry of caffeine in tea coupled with sample pretreatment using a cartridge column filled with polyvinylpyrrolidone (PVPP). Chem. Pharm. Bull. 56: 185–188.

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